

WHAT IS CLAIMED IS:

1. A method of growing human hematopoietic cells in culture, said method comprising:

inoculating a reactor vessel comprising stromal cells adherent to a protein substrate with human hematopoietic cells comprising progenitor cells, wherein at least a portion of said stromal cells are transformed fibroblast cells capable of adhering to a protein surface and capable of excreting at least one growth factor which directs the proliferation and/or differentiation of said progenitor hematopoietic cells;

substantially continuously perfusing said cells in said reactor with a nutrient medium comprising any additional growth factors necessary for proliferation and/or differentiation of said hematopoietic cells, while removing metabolic products and replenishing depleted nutrients, while maintaining said reactor under physiologically acceptable conditions; and

20 harvesting hematopoietic cells from said reactor, with the proviso that when said human hematopoietic cells inoculated into said reactor vessel are suspected of comprising neoplastic cells, said perfusing is at a rate providing a force greater than the affinity of neoplastic cells to said stromal cells and less than the affinity of normal hematopoietic cells.

25 2. A method according to Claim 1, wherein said stromal cells excrete at least one growth factor.

30 3. A method according to Claim 2, wherein at least one growth factor is human GM-CSF or IL-3.

35 4. A method according to Claim 1, wherein said perfusion rate resulting in a shear stress at the surface of the hematopoietic cells greater than about 1.0 dyne/cm<sup>2</sup>.

5. A method according to Claim 1, wherein said protein substrate is a protein coated membrane or protein sponge.
- 5 6. A method according to Claim 5, wherein said protein is collagen and/or fibronectin.
7. A method according to Claim 1, wherein said transformed cells are physically separated from said normal bone marrow cells by a physical barrier.
- 10 8. A method according to Claim 1, wherein said stromal cells are maintained prior to harvesting substantially at a subconfluent stage.
9. A method according to Claim 1, further comprising recycling hematopoietic stem cells from said nutrient medium exiting said reactor.
- 15 10. A method according to Claim 1, wherein said perfusing is at a flow rate to maintain production of hematopoietic growth factors at about the endogenous level produced by said normal bone marrow stromal cells.
- 20 11. A method according to Claim 1, wherein said perfusing with said nutrient medium and said stromal cells supports the division of human bone marrow stem cells, whereby human bone marrow stem cells are produced in said vessel, and said method further comprises transfecting said human bone marrow stem cells with a gene of interest present in a retroviral vector.
- 25 12. A method of growing human hematopoietic cells in culture, said method comprising:  
inoculating a reactor vessel comprising heterologous stromal cells adherent to one side of a protein substrate with pores in the range of about 1-5 microns with human hematopoietic cells comprising progenitor cells, said inoculation being on the opposite side of said membrane from said stromal cells, wherein at least a portion of said stromal cells are transformed fibroblast cells capable of adhering to a protein surface and capable of excreting at least one

growth factor which directs the proliferation and/or differentiation of said progenitor hematopoietic cells; substantially continuously perfusing said cells in said reactor with a nutrient medium comprising  
5 any additional growth factors necessary for proliferation and/or differentiation of said hematopoietic cells, while removing metabolic products and replenishing depleted nutrients, while maintaining said reactor under physiologically acceptable  
10 conditions; and

harvesting hematopoietic cells from said reactor,

with the proviso that when said human hematopoietic cells inoculated into said reactor vessel  
15 are suspected of comprising neoplastic cells, said perfusing is at a rate providing a force greater than the affinity of neoplastic cells to said stromal cells and less than the affinity of normal hematopoietic cells.

20 13. A method according to Claim 2, wherein said hematopoietic cells are bone marrow cells.

14. A method according to Claim 12, wherein said perfusing provides a glucose concentration in the range of about 5 to 20mM and a glutamine concentration in the range of about 1 to 3mM , while the lactate concentration is maintained below about 35mM and the ammonia concentration is maintained below about 2.5mM.  
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15. A bioreactor comprising:  
a reactor chamber;  
means for introducing and removing a nutrient medium from said reactor chamber and means for monitoring the effluent from said reactor chamber;  
in said reactor chamber, stromal cells adherent to a protein substrate with human hematopoietic cells comprising progenitor cells, wherein at least a portion of said stromal cells are transformed fibroblast  
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cells capable of adhering to a protein surface and capable of excreting at least one growth factor which directs the proliferation and/or differentiation of said progenitor cells.

5        16. A bioreactor according to Claim 15, wherein said protein substrate is a protein coated membrane with pores of a size in the range of about 1-5 microns, with said stromal cells adherent to one side of said membrane and said hematopoietic cells present on the opposite side.

10        17. A bioreactor according to Claim 15, wherein said protein substrate is protein sponge.

15        18. A bioreactor according to Claim 15, further comprising means for maintaining said stromal cells substantially at a subconfluent stage.

19. A bioreactor according to Claim 16, wherein said means for introducing and removing a nutrient medium comprises:

20        a media reservoir for storing media; means for transporting fresh media into said reservoir and removing partially spent media from said reservoir;

25        means for transporting media from said reservoir to said bioreactor and from said bioreactor to said reservoir;

means for oxygenating said media prior to introduction into said bioreactor; and

means for monitoring the composition of said media from said bioreactor.

30        20. A bioreactor reactor according to Claim 16, further comprising means for isolating hematopoietic stem cells from said exiting nutrient medium and returning said hematopoietic stem cells to said reactor chamber.

35        21. Transformed fibroblast cells comprising a DNA expression construct capable of expressing at least one

human growth factor in a form capable of excretion which growth factor directs the proliferation and/or differentiation of progenitor hematopoietic cells .

22. Transformed fibroblast cells according to  
5 Claim 21, wherein said growth factor is a colony stimulating factor or a interleukin..

23. Transformed fibroblast cells according to  
Claim 22, wherein said colony stimulating factor is GM-CSF and said interleukin is IL-3.

10 24. Transformed fibroblast cells according to  
Claim 21, wherein said DNA expression construct comprises a promoter inducible in hematopoietic cells.

25. Transformed fibroblast cells according to  
claim 21, wherein said cells are other than primate.

15 26. A method of separating hematopoietic neoplastic cells from normal cells comprising:  
combining a cell population of hematopoietic cells with stromal cells, wherein said stromal cells have limited mobility and said hematopoietic cells contact  
20 said stromal cells; and

subjecting said hematopoietic to a fluid flow producing a force at least sufficient to remove neoplastic cells from contact with said stromal cells, without significant removal of normal cells.

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